Histopathologic Changes Seen in Esophagectomy Specimens From the High-Risk Region of Linxian, China: Potential Clues to an Etiologic Exposure?

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Esophageal cancer is one of the most fatal cancers worldwide and is characterized by great variation in rates among different populations. Linxian, a county in Henan Province, located in north-central China, has one of the highest rates of esophageal squamous cell carcinoma in the world. Most squamous cell carcinomas in low-risk populations are attributable to alcohol and tobacco consumption, but the causative agents in high-risk populations are less clear. The prevention and treatment of esophageal cancer in high-risk regions, such as Linxian, are limited by our inability to identify these agent(s). During a preliminary histological review, the authors noticed characteristic findings in the arteries, nerves, and lymph nodes of esophagectomy specimens from Linxian and wondered whether these findings might offer clues to the cause of squamous cell carcinoma (eg, polycyclic aromatic hydrocarbon exposure) in the Linxian population. The purpose of this study was to report these previously undescribed histopathologic changes and to compare their presence and severity with those found in esophageal squamous cell carcinomas and adenocarcinomas from a lower-risk population in the United States. Forty esophagectomies were reviewed, including 13 squamous cell carcinomas from Linxian and 21 squamous cell carcinomas and six adenocarcinomas from the United States. The presence and severity of arteriosclerosis and myxoid degeneration of nerves and the presence of anthracosis in periesophageal lymph nodes were recorded. The prevalence and severity of these findings in the three groups of esophagectomies were compared. The esophageal squamous cell carcinomas from Linxian, China, had a higher prevalence of arteriosclerotic vessels, nerves with myxoid degeneration, and anthracotic lymph nodes than the squamous cell carcinomas from the United States (Wilcoxon test, $P < .04$ for all comparisons). There were also significant differences in the prevalence of arteriosclerotic vessels and anthracotic lymph nodes between the esophageal squamous cell carcinomas from Linxian and the adenocarcinomas from the United States. Arteriosclerosis and the myxoid degeneration were significantly more severe in the esophageal squamous cell carcinomas from Linxian than in the esophageal squamous cell carcinomas or adenocarcinomas from the United States (Mantel trend test, $P < .006$ for all comparisons). Arteriosclerotic vessels, nerves with myxoid degeneration, and anthracotic lymph nodes can be seen in association with esophageal squamous cell carcinomas from the high-risk region of Linxian, China. These changes appear to be more prevalent and severe than those seen in association with esophageal squamous cell carcinomas or adenocarcinomas from a low-risk population in the United States. These characteristic changes may be causatively significant and may represent histological evidence of high-level environmental exposure to polycyclic aromatic hydrocarbons. HUM PATHOL 29:1294-1298. Copyright © 1998 by W.B. Saunders Company

Key words: **esophagus, cancer, China, etiology, anthracosis, arteriosclerosis, coal, polycyclic aromatic hydrocarbons.**

Abbreviations: **EC, esophageal cancer; SCC, squamous cell carcinoma; ACA, adenocarcinoma; PAH, polycyclic aromatic hydrocarbons; B[a]P, benzo(a)pyrene.**

Esophageal cancer (EC) is one of the most common cancers worldwide.[1,2] Linxian, a county in Henan Province, located in north-central China, has one of the highest rates of EC in the world, with annual age-adjusted mortality rates of up to 169 per 10^5 and cumulative death rates by age 75 years of over 20% in both sexes.[3,4] Many studies of EC have been performed in Linxian over the past 40 years, but the dominant causative factors remain unclear.[4-6]

During an initial histological review of squamous cell carcinomas (SCCs) from Linxian, one of the authors (M.J.R.) noticed a high prevalence of arteriosclerotic vessels, nerves with myxoid degeneration, and anthracotic lymph nodes. If similar changes are uncommon in esophagectomies from lower-risk populations, then these changes might provide clues to the cause of EC in Linxian. The following study represents the first report of these characteristic histological findings in the esophagectomies from this high-risk region and attempts to compare the prevalence and severity of the arteriosclerotic vessels, nerves with myxoid degeneration, and anthracotic lymph nodes in esophagectomies from Linxian with those from a lower-risk population in the United States.

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0046-8177/98/2911-0019$8.00/0

MATERIALS AND METHODS

Patients and Specimen Characteristics

Linxian Cases. The 13 Linxian patients, including 10 men (77%) and three women (23%), with a median age of 55 years (range, 46 to 71), were identified during screening endoscopies to have in situ or invasive SCC and were subsequently treated by esophagectomy in 1995. The resected specimens and harvested lymph nodes were formalin fixed and then completely divided into 2.5 × 0.6-cm sections and paraffin embedded. One hematoxylin and eosin–stained slide was prepared from each section for a median of 47 slides per case (range, 28 to 56; 583 total slides). Because the cases were identified during screening endoscopies, most were early carcinomas, with 11 (85%) being TNM stage 1 or less.[7] Data on tobacco smoking and alcohol usage was not available (Table 1).

UCLA Cases. The 27 esophagectomy cases, including 21 squamous cell carcinomas and six adenocarcinomas (ACAs) from 11 men (41%) and 16 women (59%) with a median age of 68 years (range, 52 to 81), were selected from the 1982-1995 pathology files of the UCLA Center for the Health Sciences, Los Angeles, California. The ACAs had been part of another research protocol and, therefore, were fully embedded in a manner similar to the Linxian cases, for a median of 43 slides per case (range, 11 to 81; 260 total slides), but the SCCs were "blocked in" per routine hospital practice and, therefore, were less extensively sampled, for a median of 11 slides per case (range, 6 to 47; 314 total slides). Most of the UCLA cases were advanced cancers, with only a single case (1 of 20 or 5%, data missing on seven cases) being TNM stage 1 or less. Data on tobacco and alcohol usage was available on 19 (90%) of the SCCs. Thirteen (68%) of these patients used either tobacco or alcohol, 12 (63%) used both tobacco and alcohol, and five (26%) used neither tobacco or alcohol.

Histological Evaluation

Each slide was histologically reviewed for arteriosclerotic vessels and for nerves with myxoid degeneration (see description in Results). The severity of each arteriosclerotic change was classified as either mild (the plaque involved ≤¼ of the circumference of the vessel and the width of the plaque was ≤¼ of the thickness of the vessel wall), moderate (the plaque involved >¼ but ≤½ of the circumference of the vessel, or the width of the plaque was >¼ but ≤½ of the thickness of the vessel wall), or severe (the plaque involved >½ of the circumference of the vessel, or the width of the plaque was >½ of the thickness of the vessel wall). The severity of myxoid degeneration in each nerve was classified as either mild (the histopathologic change involved <¼ of the cross section of the nerve), moderate (the histopathologic change involved >¼ but ≤½ of the cross section of the nerve), or severe (the histopathologic change involved >½ of the cross section of the nerve). Periesophageal lymph nodes were histologically reviewed for the presence of anthracotic pigment.

TABLE 1. Patient and Specimen Characteristics

	Linxian SCC	UCLA SCC	UCLA ACA
No. of patients	13	21	6
Males (%)	77	38	50
Median age (yr)	55	68	62
Total No. of slides	583	314	260
Median No. of slides per case (range)*	47 (28-56)	11 (6-47)	43 (11-81)
TNM Stage ≤1†	11	0	1

*The UCLA SCCs had significantly fewer slides per case than the Linxian SCCs (Wilcoxon $P < .001$) or the UCLA ACAs ($P = .0065$).

†Stage was known for 13 Linxian SCCs; 18 UCLA SCCs; 2 UCLA ACAs.

Statistical Analysis

Differences in the prevalences of the vascular and neural changes in the three groups of esophagectomy specimens were compared using the Fisher's exact test. Adjustment for differences in the number of slides reviewed per case was done by comparing the mean percentage of slides showing these changes in each case using the Wilcoxon rank-sum test. Differences in the severity of the vascular and neural changes were tested using the Mantel trend test.[8] All *P*-values result from testing two-sided hypotheses.

RESULTS

The initial review of the Linxian esophagectomies showed three striking findings: arteriosclerosis, myxoid degeneration of nerves, and anthracotic lymph nodes. The arteriosclerotic vessels and the nerves showing myxoid degeneration were located predominantly in the adventitia of the esophagus. The arteriosclerosis was histologically similar to Monckeberg's arteriosclerosis (medial calcific sclerosis) and consisted of calcium deposits primarily involving the internal elastic lamina, without an associated intimal proliferation or inflammatory reaction (Fig 1A).[9] The myxoid degeneration was histologically similar to the myxoid change seen in some neurofibromas (Fig 1B).[10] The anthracotic lymph nodes were histologically similar to those seen in association with smoking or coal exposure, characterized by sinus histiocytosis, fibrosis, and black pigment (Fig 1C).[11]

The Linxian SCCs had significantly more cases with arteriosclerotic vessels than the UCLA SCCs ($P = .003$) or the UCLA ACAs ($P = .006$) (Table 2). The Linxian SCCs had significantly more nerves with myxoid degeneration than the UCLA SCCs ($P = .001$), but the difference between the Linxian SCCs (85%) and the UCLA ACAs (33%) was not significant ($P = .09$). All of the Linxian SCCs had either vascular or neural changes, and most (62%) had both.

The Linxian SCCs had a similar number of slides reviewed per patient as the UCLA ACAs, but the UCLA SCCs had significantly fewer slides (Wilcoxon $P < .0001$) (Table 1). Because the likelihood of finding histopathologic changes could increase with the number of slides reviewed, we adjusted for the differences in slide number by comparing the percentage of slides from each patient showing arteriosclerosis (mean [median]: Linxian SCC 4 [3]; UCLA SCC 3 [0]; UCLA ACA 0 [0]) and the percentage of slides showing myxoid degeneration of nerves (Linxian SCC 16 [9]; UCLA SCC 4 [0]; UCLA ACA 2 [0]). The higher prevalences seen in the Linxian SCCs remained significant (Wilcoxon test for arteriosclerosis, Linxian SCCs *v* UCLA SCCs $P = .0146$, Linxian SCCs *v* UCLA ACAs $P = .0062$; for myxoid

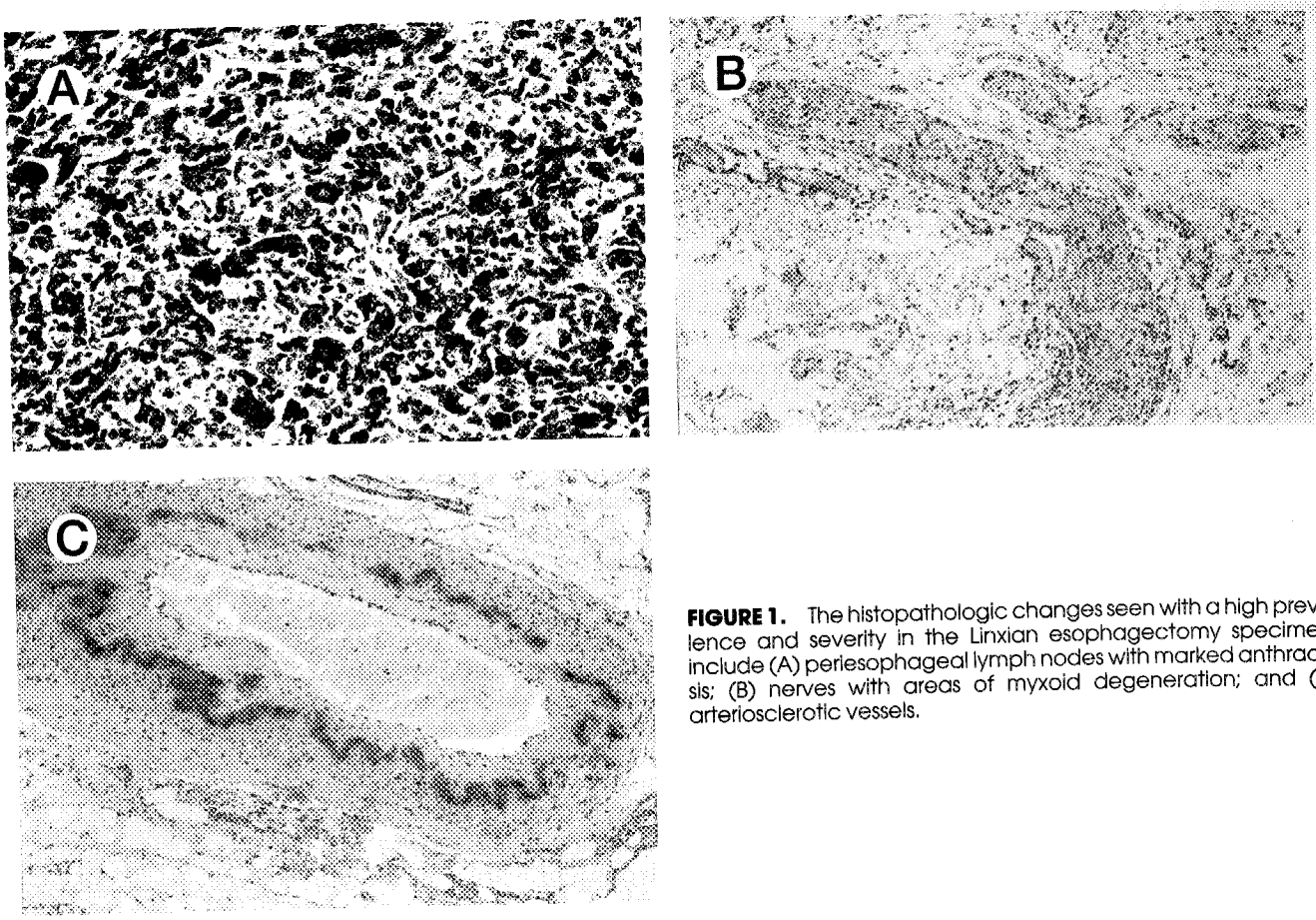

FIGURE 1. The histopathologic changes seen with a high prevalence and severity in the Linxian esophagectomy specimens include (A) periesophageal lymph nodes with marked anthracosis; (B) nerves with areas of myxoid degeneration; and (C) arteriosclerotic vessels.

degeneration of nerves, Linxian SCCs *v* UCLA SCCs $P = .0026$, Linxian SCCs *v* UCLA ACAs $P = .0086$).

The worst vascular and neural changes seen in each case were more severe in the Linxian SCCs than in the UCLA SCCs or UCLA ACAs (Mantel trend test: for arteriosclerosis, Linxian SCCs *v* UCLA SCCs $P = .0026$, Linxian SCCs *v* UCLA ACAs $P = .0059$; for nerve Linxian SCCs *v* UCLA SCCs $P = .0002$, Linxian SCCs *v* UCLA ACAs nerve $P = .0034$) (Table 3).

One or more lymph nodes were present in the esophagectomy specimens of all 13 Linxian SCCs, in 16 (76%) of the 21 UCLA SCCs, and in two (33%) of the six UCLA ACAs. At least one lymph node containing anthracotic pigment was found in 13 of 13 (100%) of the Linxian SCCs, 8 of 16 (50%) of the UCLA SCCs, and 0 of two (0%) of the UCLA ACAs (Fisher's exact test, Linxian SCCs *v* UCLA SCCs $P = .006$, Linxian SCC *v* UCLA ACAs $P = .02$). The Linxian SCCs also had a higher percentage of lymph nodes with anthracosis (median, 64%) than the UCLA SCCs (12%) (Wilcoxon test, $P = .01$).

TABLE 2. Prevalence of Vascular and Neural Changes

Study Group	Arterio-sclerotic Vessels*	Myxoid De-generation of Nerves†	Vascular OR Neural Changes‡	Vascular and Neural Changes
Linxian SCC (N = 13)	10 (77%)§	11 (85%)	13 (100%)	8 (62%)
UCLA SCC (N = 21)	4 (19%)	5 (24%)	8 (38%)	1 (5%)
UCLA ACA (N = 6)	0 (0%)	2 (33%)	2 (33%)	0 (0%)

*Significantly fewer of the UCLA SCC and UCLA ACA patients had arteriosclerotic vessels than the Linxian SCC patients, Fisher's exact Linxian SCC *v* UCLA SCC $P = .003$; Linxian SCC *v* UCLA ACA $P = .006$.

†Significantly fewer of the UCLA SCC patients had myxoid degeneration of nerves than the Linxian SCC patients, Fisher's exact $P = .001$. The difference was not significant for Linxian SCC *v* UCLA ACA, $P = .09$.

‡Significantly fewer of the UCLA SCC and UCLA ACA patients had either vascular or neural changes than the Linxian SCC patients, Fisher's exact Linxian SCC *v* UCLA SCC $P = .00006$; Linxian SCC *v* UCLA ACA $P = .00774$.

§No. of cases (row percent).

TABLE 3. Severity of Vascular and Neural Changes

Study Group	No Change	Mild	Moderate	Severe
Linxian SCC ($N = 13$)				
Arteriosclerosis*	3 (23%)‡	2 (15%)	1 (8%)	7 (54%)
Neural change†	2 (15%)	0 (0%)	5 (38%)	6 (46%)
UCLA SCC ($N = 21$)				
Arteriosclerosis*	17 (81%)	1 (5%)	0 (0%)	3 (14%)
Neural change†	16 (76%)	1 (5%)	3 (14%)	1 (5%)
UCLA ACA ($N = 6$)				
Arteriosclerosis*	6 (100%)	0 (0%)	0 (0%)	0 (0%)
Neural change†	4 (67%)	2 (33%)	0 (0%)	0 (0%)

*The worst arteriosclerotic vessels in the Linxian SCCs were more severely affected than those in both the UCLA SCCs (Mantel trend test $P = .026$) and the UCLA ACAs ($P = .0059$).

†The worst myxoid degeneration of nerves in the Linxian SCCs was more severe than that in both the UCLA SCCs (Mantel trend test $P = .0002$) and the UCLA ACAs ($P = .0034$).

‡Number of cases (row %).

DISCUSSION

Esophageal cancer continues to be a significant cause of mortality worldwide.[1] Several risk factors have been identified, including tobacco smoking, alcohol consumption, gender, race, and diet.[12] However, despite our current understanding of the nature of EC, there remain high-risk regions in the world, including China, Iran, and several central Asian states, in which the major causative agents are still unknown.[13] The identification of causative agents in these high-risk regions may help to advance the development of effective prevention and treatment methods. During an initial histological review of esophagectomy specimens from Linxian, China, unexpectedly we found arteriosclerotic vessels, nerves with myxoid degeneration, and lymph nodes showing anthracotic pigment that appeared to be far in excess of such changes seen in esophageal squamous cell carcinomas from the United States.

This study was potentially limited by differences in the case populations between the two groups. These include differences in (1) gender distribution, (2) median patient age, (3) the stage distribution of the tumors, (4) the number of slides reviewed, and, possibly, (5) smoking and alcohol consumption. These differences do not appear, however, to have significantly biased our results. The differences in prevalence of the vascular, neural, and lymph node changes between the male and females of each study group were not significant (Fisher's exact $P > .10$ for all comparisons, data not shown). The age and stage differences between the two groups ran counter to what one would expect: though more severely affected by vascular, neural, and lymph node changes, the Linxian cases were both younger and at an earlier cancer stage than those from UCLA. The differences in prevalence remained significant after adjusting for differences in number of slides and finally, although data for the Linxian cases on tobacco smoking and alcohol consumption was not available, they probably experienced these harmful exposures less than their Western counterparts: studies have shown only mild tobacco usage and minimal alcohol consumption in the Linxian population,[6] but 74% of the UCLA SCC patients were smokers or drinkers, and 63% were both. Thus, despite the differences in the case populations, we think that our findings represent real differences in the prevalence and severity of these histolopathologic features.

There is evidence to suggest that the high prevalence and severity of the histopathologic changes seen in the Linxian specimens could be causatively related to this region's high rate of EC. Lymph node anthracosis is frequently associated with smoking or environmental pollution.[11] It seems unlikely that smoking is the major contributing factor causing the anthracosis; however, because all of the female Chinese patients had anthracotic lymph nodes, even though in Linxian nearly all the smoking is done by men.[4,6] A more likely explanation is environmental pollution. Until recently, the Linxian region was completely rural, with no source of industrial pollution. Vehicle exhaust was also minimal; there were only a few tractors and buses, and no cars. The most likely source of environmental pollution over the life span of these patients has been the use of coal in homes for heating and cooking. The inhabitants of this region use coal mixed with mud to form bricks that are placed in small, typically unvented, centrally placed stoves that are used daily for cooking and heating. This practice results in high-level exposure to the incomplete combustion products of coal, including carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (B[a]P).[5] Several environmental studies have associated PAH exposure with the development of cancers of the esophagus, stomach, and lung,[14-22] and a recent 2-year feeding study in mice conducted by the National Center for Toxicological Research identified a striking dose-response relationship between B[a]P food levels and the incidence of squamous cell carcinoma of the esophagus.[23] In addition, we recently have found high levels of B[a]P in staple foods from Linxian.[24] Thus, the high prevalence of anthracotic lymph nodes in the Linxian SCCs may be related to chronic inhalation and ingestion of coal dust and smoke that are present in the air and coating the food and, therefore, may be a histopathologic marker of high-level exposure to environmental carcinogens such as PAHs.

There is also evidence to associate chronic inhalation and ingestion of coal dust and smoke, and consequently, high-level PAH exposure, with the high prevalence and severity of the vascular changes found in the Linxian SCCs. PAH metabolites can induce vascular cell metabolism of B[a]P to carcinogenic and toxic products that accelerate atherosclerosis, and DNA adducts, a marker of PAH exposure, can be detected in atherosclerotic lesions.[25-27] The causative significance of the high prevalence and severity of the myxoid degeneration of nerves found in the Linxian SCCs is less clear because there are no studies to associate myxoid degeneration of nerves with exposure to high levels of environmental carcinogens, such as PAHs. The myxoid changes may present a possible clue to another as yet undefined exposure.

In summary, this study found characteristic vascular, neural, and lymph node changes that were common and severe in Linxian esophagectomy specimens and uncommon and less severe in US esophagectomies. These changes may be secondary to the Linxian region's extensive use of coal and may represent histological evidence of high-level environmental exposure to PAHs that may be associated with the high rate of EC in Linxian. This hypothesis deserves further study.

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